

REMARKS

Applicants acknowledge the Examiner's statement that "Claims 89-99 are free of prior arts".

THE AMENDMENTS

Applicants cancel all pending claims, claims 61-99, without prejudice, and add new claims 100-127. Applicants reserve the right to prosecute cancelled claims and other claims based on their subject matter in this or other applications. New claims 100-127 are based on canceled claims 89-99. These claims add no new subject matter, and are fully supported throughout the specification and by the drawings and claims as filed. Support and reasoning for the amendments are provided below.

Support for New Claims and Reasons for Amendments

These amendments are made to clarify the claims in order to expedite allowance of the present application.

New claim 100, which recites a method of separating one or more moieties from a blood sample, is based on canceled claim 89. Support for new claim 100 can be found in filed claims 44, 7, and 1. Support for claim 100, and also dependent claim 104, which specifies an osmolarity of the sample after the addition of the solution of from about 30 mOsm to 100 mOsm, can also be found in the specification, page 35, lines 10-31:

Certain preferred embodiments of the present invention are sample solutions having a low conductivity that, when combined with a blood sample, selectively modify red blood cells such that they are not retained in a chamber subject to dielectrophoretic forces. In these preferred embodiments, a sample solution of the present invention preferably modifies a higher percentage of red blood cells than white blood cells, hereinafter referred to as a solution that selectively lyses red blood cells. Preferably, mixing a preferred solution of the present invention with a blood sample results in the blood sample having a ratio of intact red blood cells to intact white blood cells that is less than 20 : 1, more preferably less than 10 : 1, and most preferably less than 5:1. This can be observed microscopically. Preferred sample solutions of the present invention can be mixed with a blood sample at a ratio of from about 1:10 to about 10,000:1, preferably at a ratio from about 1: 5 to about 1000: 1, more preferably at a ratio from about 1: 1 to about 250: 1, and most preferably at a ratio from about 2: 1 to about

50: 1. These preferred sample solutions of the present invention have a low conductivity, meaning that their polarizability in response to an applied electric field is less than that of the moieties to be separated. Preferably, the conductivity of a sample solution of a preferred embodiment of the present invention is between about 10 mOsm and about 250 mOsm, more preferably between about 20 mOsm and about 150 mOsm, and most preferably between about 30 mOsm and about 100 mOsm.

In certain aspects of these preferred embodiments, a solution of the present invention preferably has a low osmolarity such that when added to a blood sample, the blood cells are in a hypotonic medium. In these embodiments, the final osmolarity is preferably between 20 mOsm and about 150 mOsm, most preferably between 30 mOsm and about 100 mOsm.

New claims 101 to 127 are dependent on claim 100. Support for claims 101, 102, and 103, referring to the conductivity of the sample-sample solution mixture, can be found on page 24 of the specification, lines 9-12:

Sample-sample solution mixtures of the present invention preferably have conductivities of between about 1 microSiemens/cm to about 1 Siemen/m, more preferably from about 5 microSiemens/cm to about 0.5 Siemens/m, and most preferably from about 10 microSiemens/cm to about 0.1 Siemens/m.

Support for claim 104, which specifies an osmolarity of from about 30 mOsm to 100 mOsm of the sample after the addition of the solution that selectively lyses red blood cells, can also be found in the specification, page 35, lines 24-27, reproduced above in the first citation supporting claim 100.

Support for claims 105-107, reciting the ratio of intact red blood cells to intact white blood cells after the addition of the solution that selectively lyses red blood cells can be found on page 49, lines 14-19:

A solution of the present invention that selectively lyses red blood cells can be any solution that when mixed with a blood sample, lyses a higher proportion of red blood cells than white blood cells. Preferably, mixing a solution that selectively lyses red blood cells with a blood sample results in the blood sample having a ratio of intact red blood cells to intact white blood cells that is less than 20 : 1, more preferably less than 10 : 1, and most preferably less than 5:1.

Support for claims 108-111, drawn to the ratio of sample buffer added to a sample, can be found in the specification on page 49, lines 19-22:

Preferred solutions of the present invention can be mixed with a blood sample at a ratio of from about 1:10 to about 10,000:1, preferably at a ratio from about 1:5 to about 1000: 1, more preferably at a ratio from about 1: 1 to about 250: 1, and most preferably at a ratio from about 2: 1 to about 50: 1.

Claims 112-117 are drawn to the composition of the sample solution. Claim 112 recites a solution comprising glycerol, one or more sugars, one or more sugar alcohols, or one or more zwitterions or zwitterionic compounds. Support for claims 112, 113, 115, and 116 is found on page 35, line 28 to page 36, line 12 of the specification:

In certain aspects of these preferred embodiments, a solution of the present invention preferably has a low osmolarity such that when added to a blood sample, the blood cells are in a hypotonic medium. In these embodiments, the final osmolarity is preferably between 20 mOsm and about 150 mOsm, most preferably between 30 mOsm and about 100 mOsm. Suitable solutes for use in low osmolarity solutions of the present invention include glycerol, sugars such as sucrose, dextrose, and mannose, and sugar alcohols such as mannitol and sorbitol. Other solutes that can be used in low osmolarity solutions of the present invention include zwitterions that have no charge at or near neutral pH, for example, glycine, alanine, gamma-amino-butyric acid, cysteine, histidine (including D-, L-, 3(J) methyl- and 1 (B) methyl- histidine), carnosine, pyridine, imidazole, and collidine (see, for example, Edman, et al. Nucl. Acids Res. 25: 4907-4914 (1997)), and zwitterionic compounds and buffering agents, such as, but not limited to, *N*-2-Acetoamido-2-amino-ethanesulfonic acid (ACES), *N,N*-Bis (2-hydroxyethyl)-2-glycine (bicine), *N*-2-Hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid (HEPES), 3-(*N*-Morpholino)-propanesulfonic acid (MOPS), piperazine-*N,N'*-bis-2-ethanesulfonic acid (PIPES), *N*-Tris(hydroxymethyl)-methyl-2-aminoethane-sulfonic acid (TES), *N*-Tris(hydroxymethyl)-methylglycine (Tricine), or Tris(hydroxymethyl)-aminomethane (Tris).

Support for claim 114, which recites a final concentration of glycerol in the sample after the addition of sample solution, can be found in Example 1, on page 62 of the specification, lines 16-19:

Other types of low osmolality RBC-lysis solutions, including various concentration of glycerol at 0.5%, 0.65%, 0.7%, 0.75%, 0.8% and 0.85% (weight to weight), were tested. For a dilution ratio 1:9 for blood to glycerol solution, 0.8% glycerol yielded the best result in terms of number of remaining white blood cells and white-blood-cell to red-blood-cell ratio.

Support for claim 117, which is drawn to the final concentration of sucrose in the sample after the addition of sample solution, can be found in Example 1, Table 1 (page 59), Table 2 (page 60), Table 3 (page 61), and Table 4 (page 62).

New claims 118-120, referring to the sample components that can be separated, are based on canceled claims 90-92, and correspond to originally filed claims 45-47.

New claim 121 is supported on page 53 of the application, lines 8-10: “Separations with electromagnetic particles are performed on electromagnetic chips, where the source of the electromagnetic force is in part separate from the chip and in part integral to the chip.” Claims 122 and 123, concerning specific binding members of the magnetic microparticles, correspond to canceled claims 94 and 95, and to originally filed claims 52 and 54.

New claims 124 and 125, regarding the composition and dimensions of the microparticles, correspond to canceled claims 96 and 97 and to originally filed claims 57 and 58.

New claims 126 and 127, regarding the order of adding the sample solution to the sample, correspond to 98 and 99, and to originally filed claims 59 and 60.

CLAIMS ARE DEFINITE UNDER 35USC §112, SECOND PARAGRAPH

The Examiner alleges that claims 61-99 are indefinite under 35 USC §112, Second Paragraph in the use of the term “selectively modifies” in Claim 61, and in the use of the term “selectively lyses” in Claim 64. Applicants have canceled claims 61-99 and presented new claims 100-127. The new claims do not recite the term “selectively modifies”. New claim 100, which is based on canceled claim 89, uses the phrase “a solution that selectively lyses red blood cells”. Applicants disagree that the phrase is indefinite. Applicants assert that this phrase is clear and definite, and has ample support in the specification. For example, on page 49, lines 14-19, the specification reads:

A solution of the present invention that selectively lyses red blood cells can be *any solution that when mixed with a blood sample, lyses a higher proportion of red blood cells than white blood cells*. Preferably, mixing a solution that selectively lyses red blood

cells with a blood sample results in the blood sample having a ratio of intact red blood cells to intact white blood cells that is less than 20 : 1, more preferably less than 10 : 1, and most preferably less than 5:1. This can be observed microscopically. [italics added]

The Examiner asks in the Office Action dated November 18, 2003 whether the solution lyses certain kinds of red blood cells, and if so, what are the kinds of red blood cells that are being lysed. Applicants respond that, as is clearly set forth in the passage cited immediately above, the term “selectively lyses red blood cells” does not mean that subsets of red blood cells are lysed preferentially. Rather, the term “selectively lyses” refers to the fact that, following the addition of a solution of the present invention to a blood sample, the majority of red blood cells are lysed, whereas white blood cells are largely left intact.

On page 14 the specification provides the accepted definition for a red blood cell: ‘A “red blood cell” is an erythrocyte.’, and on the same page provides a definition for a white blood cell: ‘A “white blood cell” is a leukocyte, or a cell of the hematopoietic lineage that is not a reticulocyte or platelet and that can be found in the blood of an animal. Leukocytes can include lymphocytes, such as B lymphocytes or T lymphocytes. Leukocytes can also include phagocytic cells, such as monocytes, macrophages, and granulocytes, including basophils, eosinophils and neutrophils. Leukocytes can also comprise mast cells.’

Applicants point out that Example 1 of the application describes testing several solutions for their capacity to lyse red blood cells while leaving the majority of white blood cells intact. Comparisons of the numbers and/or conditions of red blood cells (RBCs) versus white blood cells (WBCs) after using adding candidate solutions to blood samples are displayed in Tables 1, 2, 3, and 4 (pages 59-62 of the specification).

Applicants therefore assert that new Claim 100, and new Claims 101-127 that are dependent on Claim 100, are definite, and respectfully request that the rejection be removed.

CLAIMS ARE NOVEL UNDER 35 USC §102

The Examiner has rejected claims 61, 62, 64, 65, 66, 70, 74-76 under 35 USC §102(e) as allegedly anticipated by Ryan (US 6,200,500). Applicants have canceled claims 61-99, reserving the right to prosecute those claims in this or future applications, and have provided new claims 100-127. Applicants therefore respectfully request that the rejection be removed.

The Examiner has also rejected claims 61, 63, 70-75, 77, 78, and 88 under 35 USC §102(e) as allegedly anticipated by Becker et al. (US 5,993,630). Applicants have canceled claims 61-99, reserving the right to prosecute those claims in this or future applications, and have provided new claims 100-127. Applicants therefore respectfully request that the rejection be removed.

The Examiner has rejected claims 61, 63, 70-74, 79-82, and 84-87 under 35 USC §102(e) as allegedly anticipated by Cheng et al. (US 6,280,590). Applicants have canceled claims 61-99, reserving the right to prosecute those claims in this or future applications, and have provided new claims 100-127. Applicants therefore respectfully request that the rejection be removed.

The Examiner has also rejected claims 61-63 under 35 USC §102(b) as allegedly anticipated by Products for Molecular Biology, Molecular Sigma Biology 1992, pp. 83-86. Applicants have canceled claims 61-99, reserving the right to prosecute those claims in this or future applications, and have provided new claims 100-127. Applicants therefore respectfully request that the rejection be removed.

CLAIMS ARE NONOBVIOUS UNDER 35 USC §103

The Examiner has rejected claims 67 and 68 under 35 USC §103(a) as allegedly unpatentable over Ryan (US 6,200,500) in view of Horan et al. (US 4,783,401). Applicants have canceled claims 61-99, reserving the right to prosecute those claims in this or future applications, and have provided new claims 100-127. Applicants therefore respectfully request that the rejection be removed.

The Examiner has rejected claim 83 under 35 USC §103(a) as allegedly unpatentable over Cheng et al. (US 6,280,590). Applicants have canceled claims 61-99, reserving the right to prosecute canceled claims in this or future applications, and have

provided new claims 100-127. Applicants therefore respectfully request that the rejection be removed.

Applicants submit that the claims are ready for examination and in condition for allowance.

Respectfully submitted,

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In the event this paper is deemed not timely filed the applicants hereby petition for an appropriate extension of time. The fee for this extension may be charged to Deposit Account No. 501,321 along with any other additional fees which may be required with respect to this paper; any overpayment should be credited to the account. If any fees charged to this account will exceed \$500, applicants respectfully requests that its counsel be notified of such amounts before the Deposit Account is charged.